



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Environmental regulation of the fim switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media

Citation for published version:

Gally, D, Bogan, JA, Eisenstein, BI & Blomfield, IC 1993, 'Environmental regulation of the fim switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media', *Journal of Bacteriology*, vol. 175, no. 19, pp. 6186-93.

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Journal of Bacteriology

Publisher Rights Statement:

Copyright © 1993, American Society for Microbiology

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Environmental Regulation of the *fim* Switch Controlling Type 1 Fimbrial Phase Variation in *Escherichia coli* K-12: Effects of Temperature and Media

DAVID L. GALLY, JACQUELINE A. BOGAN, BARRY I. EISENSTEIN,[†]
AND IAN C. BLOMFIELD*

Department of Microbiology and Immunology, Bowman Gray School of Medicine,
Wake Forest University, Medical Center Boulevard, Winston-Salem,
North Carolina 27157-1064

Received 12 May 1993/Accepted 22 July 1993

Expression of type 1 fimbriae in *Escherichia coli* K-12 is phase variable and associated with the inversion of a short DNA element (switch). The *fim* switch requires either *fimB* (on-to-off or off-to-on switching) or *fimE* (on-to-off switching only) and is affected by the global regulators leucine-responsive regulatory protein (Lrp), integration host factor (IHF), and H-NS. Here it is shown that switching frequencies are regulated by both temperature and media and that these effects appear to be independent. *fimE*-promoted on-to-off switching occurs far more rapidly than previously estimated (0.3 per cell per generation in defined rich medium at 37°C) and faster at lower than at higher temperatures. In direct contrast, *fimB*-promoted switching increases with temperature, with optima between 37 and 41°C. Switching promoted by both *fimB* and *fimE* is stimulated by aliphatic amino acids (alanine, isoleucine, leucine, and valine), and this stimulation requires *lrp*. Furthermore, *lrp* appears to differentially regulate *fimB*- and *fimE*-promoted switching in different media.

Type 1 fimbriae are filamentous proteinaceous appendages produced by many species of enteric bacteria. Type 1 fimbriae promote attachment to a variety of eukaryotic cells by a process inhibited by mannose (24). Recent evidence suggests that type 1 fimbriae play an important role in communicability (3). The expression of type 1 fimbriae may play a role in urinary tract infections (24). However, type 1 fimbriae are excellent immunogens (10, 28, 39, 40). Thus, the capacity to switch rapidly their expression off or on, particularly in response to specific signals, should be advantageous to the organism and consequently important in pathogenesis.

Type 1 fimbrial phase variation is associated with the inversion of a 314-bp DNA element (1). This switch (invertible element) contains a promoter for *fimA*, the main fimbrial structural subunit gene (16). Thus, *fimA* is expressed in one orientation (on) but not the other (off). Switching is RecA independent (site specific) and requires either *fimB* (on-to-off or off-to-on switching) or *fimE* (on-to-off switching only) (6, 17, 27, 32, 33, 38). In addition to *fimB* and *fimE*, genes situated adjacent to the switch, switching is also influenced by at least three global regulators, leucine-responsive regulatory protein (Lrp), H-NS, and integration host factor (IHF) (4, 11, 15, 21, 26, 45).

Recently, we and others have shown that many K-12 strains studied are *fimE* mutants and that slow switching results from these mutations (6). Preliminary analysis of *fimE*⁺ K-12 strains demonstrated that on-to-off switching is much faster (at least 0.01 per cell per generation) than *fimB*-promoted switching (10⁻³ to 10⁻⁴) (4, 6). Mutations in *lrp* (Lrp) and *himA* and *himD* (IHF) markedly reduce both *fimB*- and *fimE*-promoted switching (4, 11, 15). In contrast, *fimB*-promoted switching is stimulated in an *hns* mutant

background (21, 26, 45). Interestingly, others have noted heterogeneity in control of phase variation among clinical isolates (2, 18, 22, 23, 41, 42) presumably reflecting differences in the genetic compositions of these strains.

The *fim* switch in *E. coli* K-12 is considered slow and random, although early work by Brinton (8) demonstrated a temperature effect in a slowly switching (10⁻⁴) strain of *E. coli* B. It has been known for many years that certain growth conditions favor the isolation of fimbriate bacteria (growth in static broth, anaerobic growth), whereas other conditions favor afimbriate bacteria (exponential growth in well-aerated broth, growth on agar) (12, 29, 35, 36). It is also clear that strong selective pressures operate under these conditions (14, 35, 36). Whether these observations reflect selective outgrowth, regulation, or a combination of these factors is unclear.

The work presented here shows that the *fim* switch is subject to environmental control, being regulated by temperature and independently by media. At temperatures typically encountered outside the mammalian host, synthesis of fimbriae should be strongly repressed. The highest probability of switching from off to on occurs at mammalian body temperature, between 37 and 41°C, probably in association with other specific signals. Much of the difference in switching frequencies among media can be attributed to Lrp and its interaction with alanine, leucine, and isoleucine plus valine.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Bacterial strains and bacteriophage are listed in Table 1; all strains are derivatives of *E. coli* K-12. Media included L broth, which consisted of (per liter) 5 g of sodium chloride, 5 g of yeast extract, and 10 g of tryptone (Difco Laboratories, Detroit, Mich.), and L agar, which consisted of L broth containing 1.5% agar (BBL, Cockeysville, Md.). MOPS [3-(*N*-morpholino)propanesulfonic acid] minimal or defined rich me-

* Corresponding author.

[†] Present address: Lilly Research Laboratories, Indianapolis, IN 46285.

TABLE 1. Bacterial strains and bacteriophage

Strain or bacteriophage	Genotype or phenotype	Reference, construction, or source
MG1655	$\lambda^- F^- Fim^+$	20
AAEC198A	MG1655 $\Delta lacZYA fimA-lacZYA$	4
AAEC370A	MG1655 $\Delta lacZYA fimA-lacZYA fimE-am18$ (switch starts on)	4
AAEC372A	MG1655 $\Delta lacZYA fimA-lacZYA fimE-am18$ (switch starts off)	4
AAEC374A	MG1655 $\Delta lacZYA fimA-lacZYA fimB-am6 fimE-am18$ (switch locked on)	4
AAEC376A	MG1655 $\Delta lacZYA fimA-lacZYA fimB-am6 fimE-am18$ (switch locked off)	4
BGEC053	AAEC198A $\Delta lacZYA fimA-lacZYA$ insert 2 (<i>lrp</i> ::mTn10)	P1 transduction of insert 2 (<i>lrp</i> ::mTn10) (4) into AAEC198A
BGEC081	AAEC372A $\Delta lacZYA fimA-lacZYA fimE-am18$ insert 2 (<i>lrp</i> ::mTn10)	P1 transduction of insert 2 (<i>lrp</i> ::mTn10) (4) into AAEC372A
Bacteriophage Plvir		Laboratory collection

dium was used for liquid growth experiments in which switching frequencies were determined (34). MOPS media were supplemented with 10 μ M thiamine and either 0.4% glucose (defined rich and minimal media) or 0.4% glycerol (minimal medium). Where indicated, the following amino acids were added to minimal MOPS medium at concentrations used in defined rich medium (34): alanine (0.8 mM), isoleucine (0.4 mM), leucine (0.8 mM), and valine (0.6 mM). Medium supplements include D-glucose, glycerol, L-alanine (A), L-isoleucine, (I), L-leucine (L), and L-valine (V). For growth on agar media, 1.5% agar was added to MOPS media. Indicator media were minimal glucose plates supplemented with X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside; BRL) at 40 μ g/ml and lactose-MacConkey agar (50 g/liter; Difco). Liquid cultures were aerated at the indicated temperature, and growth was monitored spectrophotometrically at 420 nm. Bacteriophage P1 transduction was carried out by standard techniques (44).

Assay of the *fim* switch. To measure switching frequencies, an assay is required to monitor the proportion of switch-on to switch-off cells in a population. In *fimE* mutants, *fimB*-promoted switching occurs slowly (10^{-3} to 10^{-4} per cell per generation) and can be measured by using a *fimA-lacZYA* fusion grown on lactose-MacConkey agar. A switch-on bacterium will give rise to a colony containing predominately switch-on cells; the converse is true for a bacterium starting with the switch in the off orientation.

Lac⁺ and Lac⁻ phase variants of *fimB⁺ fimE⁺ fimA-lacZYA* (AAEC198A) cannot be detected on MacConkey agar at 37°C because of rapid on-to-off switching promoted by *fimE* (6). However, plating this strain onto prewarmed glucose minimal MOPS agar plus X-Gal at 42°C slows switching enough to produce phase variants (Fig. 1).

Determination of switching frequencies in liquid media. To measure switching frequencies, a predominately switch-on or switch-off inoculum is required. After inoculation into liquid culture, and ideally after both exponential and balanced growth is attained, the proportion of switch-on to switch-off cells in the culture is monitored. A switch-on colony, isolated on minimal MOPS agar plus X-Gal at 42°C, was plugged and inoculated into the required growth medium at 42°C. After a few generations of growth, this starter culture was used to inoculate the same medium at test temperatures. Samples were taken at timed intervals, diluted, and plated onto the appropriate assay medium. By using this protocol, the major transition is a temperature downshift and not an agar to liquid medium or minimal to rich medium change.

The overriding on-to-off switching promoted by *fimE* prevents accurate determination of *fimB*-promoted switching frequencies in a wild-type (*fimE⁺*) background. In order to measure *fimB*-promoted switching, *fimB⁺ fimE⁺ fimA-lacZYA* strains AAEC370A (switch starts on) and AAEC372A (switch starts off) were used. Measurements were made with single cell inocula (37).

Measurement of switching frequencies on agar media. Switch-on and switch-off colonies from minimal glucose-X-Gal agar were diluted and plated onto the appropriate agar media at the required temperature. The subsequent colonies were diluted and assayed for the proportion of switch-on to switch-off bacteria (13).

Calculation of switching frequencies. Switching frequencies for growth on agar and for *fimB*-promoted switching in liquid (when single cell inocula were used) were calculated by using equation 1:

probability of switching (per cell per generation)

$$= 1 - \sqrt[n]{1-x} \quad (1)$$

where n = number of generations. When measuring off-to-on frequencies from an off inoculum, x = (number of on colonies)/(total number counted). When measuring on-to-off frequencies from an on inoculum, x = (number of off colonies)/(total number counted).

To measure the faster *fimE*-promoted, on-to-off switching frequencies in liquid media, equation 2 was used. Equation 2 takes into account switching in both directions:

proportion of switch-on bacteria

$$= P \times V^n + (K_1 \times \Sigma V^{n-1}) \quad (2)$$

where K_1 = *fimB*-promoted off-to-on switching frequency (per cell per generation), K_2 = *fimB*-promoted on-to-off switching frequency, K_3 = *fimE*-promoted on-to-off switching frequency, $V = 1 - (K_2 + K_3)$, P = proportion of switch-on bacteria in inoculum, and n = number of generations.

The line of best fit to the experimental data was determined from modeled switching curves. Curve fitting was carried out with a Microsoft Excel spread sheet. Graphs for the changing proportions of switch-on and switch-off bacteria could be generated for any combination of on-to-off and off-to-on switching frequencies.

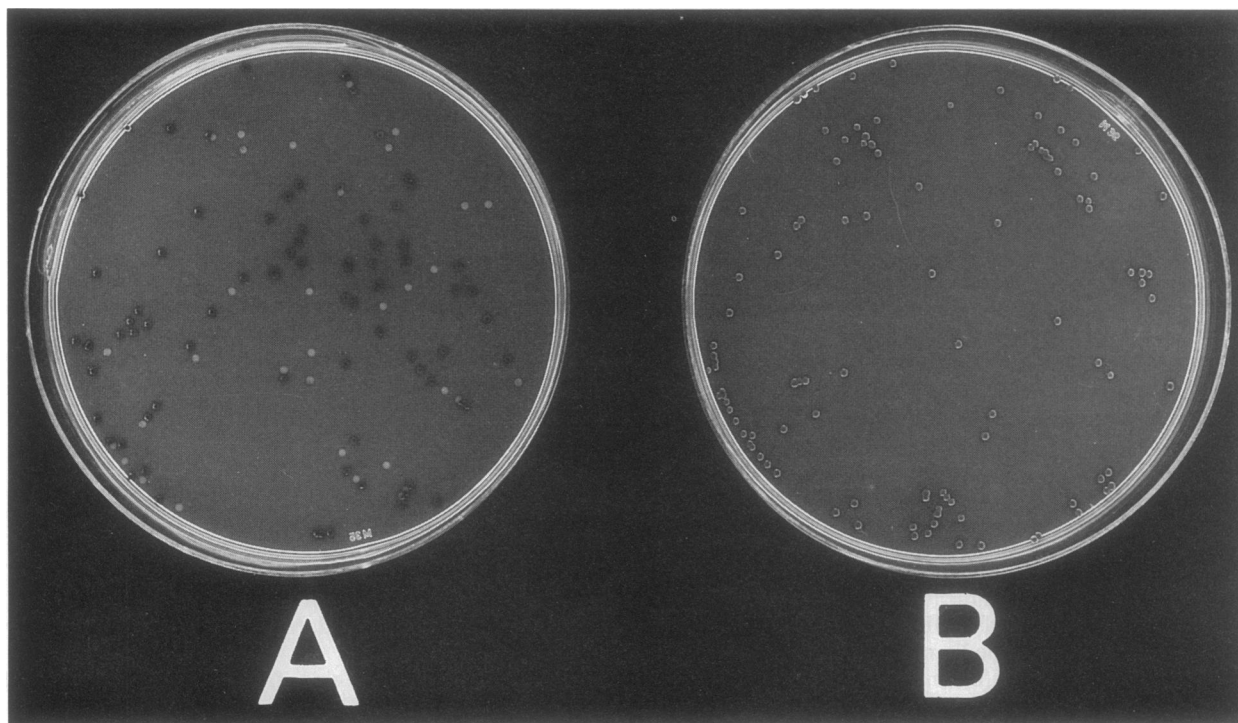


FIG. 1. Assay of the proportion of switch-on and switch-off bacteria. AAEC198A (*fimB*⁺ *fimE*⁺ *fimA-lacZYA*) grown on MOPS minimal glucose agar with X-Gal (40 μ g/ml) at 42°C (A) and 28°C (B). Phase-variant colonies were detectable only at the higher temperature.

RESULTS

Switching frequencies on agar. Earlier studies of the *fim* switch, employing *fimE* mutants, were simplified by using *fimA-lacZYA* transcriptional fusions (13, 37). In these mutants, slow switching allowed the formation of phase-variant colonies on lactose-MacConkey indicator medium at 37°C. Under the same conditions, the wild-type AAEC198A (*fimE*⁺) produces uniform colonies. In the wild type, rapid on-to-off switching ensures that colonies attain equilibrium, irrespective of the starting orientation of the *fim* switch (6).

To determine whether phase-variant colonies could be obtained with the wild type under modified conditions, AAEC198A was grown on various agar media and at different temperatures. Growth on either minimal MOPS-glucose or glycerol-X-Gal agar at 37°C produced phase variants. However, phase variants were detectable only as microcolonies at 37°C, whereas growth at 42°C produced clear phase variants (Fig. 1). These initial observations (i) showed that both media and temperature affect switching and (ii) permitted the development of a simple plate assay to determine switching frequencies (see Materials and Methods).

Switching frequencies were subsequently determined for growth on different agar media at 28, 37, and 42°C (Table 2). On-to-off switching on minimal agar was slower than on rich agar (two- to threefold) and was further reduced by growth at 42°C compared with 37°C. Growth on minimal medium at higher temperatures slows the on-to-off switching from >0.1 (rich agar medium at 37°C) to 8×10^{-3} (minimal glucose medium at 42°C). Switching was slower at 42°C than at 28°C (Fig. 1). Increased on-to-off switching at 28°C prevents the formation of phase-variant colonies.

Switching frequencies in liquid media: *fimB*⁺ *fimE*⁺. The heterogeneous nature of colony growth complicates analysis

of factors affecting the *fim* switch. Furthermore, rapid switching frequencies (greater than 0.1 per cell per generation) cannot be measured. In contrast, growth in liquid medium allows rapid switching to be measured under controlled conditions during exponential growth.

In defined rich medium at 37°C, on-to-off switching was remarkably rapid (0.3 per cell per generation; Fig. 2A). This is 30-fold faster than estimated previously (4, 6) and 300-fold faster than in *fimE* mutants (4). In this medium, switching was responsive to temperature (Fig. 2A and Table 3). On-to-off switching (dominated by *fimE*) declined with in-

TABLE 2. Switching frequencies of AAEC198A (*fimB*⁺ *fimE*⁺ *fimA-lacZYA*) at different temperatures on agar

Agar medium and type of switching	Switching frequency ^a (10^{-4}) at:		
	28°C	37°C	42°C
Defined rich			
On to off	>1,000	>1,000	1,000 \pm 100
Off to on	12 \pm 8 ^b	21 \pm 5	6.3 \pm 4
Minimal + glucose			
On to off	NT ^c	420 \pm 300	80 \pm 66
Off to on	NT	29 \pm 10	5.4 \pm 3
Minimal + glycerol			
On to off	NT	330 \pm 160	70 \pm 30
Off to on	NT	9.8 \pm 4	11 \pm 8

^a Switching frequencies (per cell per generation) were determined by using equation 1 (see Materials and Methods).

^b 95% confidence interval calculated by using the *t* distribution.

^c NT, not tested.

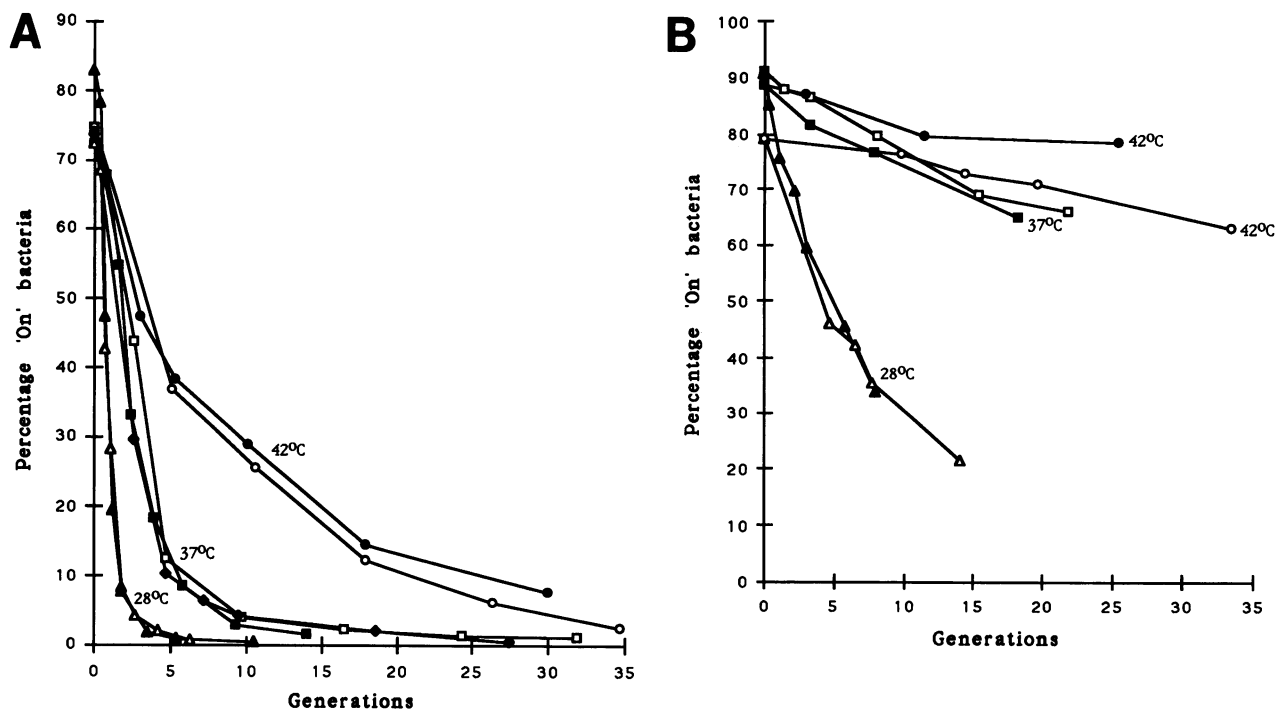


FIG. 2. On-to-off switching of AAEC198A (*fimB*⁺ *fimE*⁺ *fimA-lacZYA*) with temperature in defined-rich medium (A) or minimal medium (B). The frequencies determined from these data are shown in Table 3.

creasing temperature; turnoff was 10 times more rapid at 28°C than at 42°C (Table 3).

On-to-off switching in minimal medium was also determined (Fig. 2B and Table 3). Switching frequencies were significantly slower in minimal than in rich medium (>10-fold). Nevertheless, switching remained responsive to temperature. Changes in the frequency of on-to-off switching with temperature in rich and minimal media paralleled one another (Fig. 3). Thus, the influences of temperature and media on the *fim* switch appear to be independent.

Under conditions of the most rapid on-to-off switching (switching at 28°C in rich medium), the culture fails to reach exponential growth before the switch achieves equilibrium. Therefore, it is not possible to distinguish whether the effect is due to the temperature shift or is a result of growth at the lower temperature. However, this problem was not encountered during growth in minimal medium, in which switching still occurred at different frequencies after exponential growth was attained. Thus, we consider the frequencies obtained to be a true reflection of switching at different temperatures.

Analysis of fimbrial phase variation is inevitably complicated by strong selection for or against fimbriate bacteria.

TABLE 3. Wild-type (*fimE*-dominated) on-to-off switching frequencies at different temperatures in liquid media

Medium	Switching frequency ^a at:		
	28°C	37°C	42°C
Defined rich	>0.75	0.3	0.085
Minimal + glucose	0.07	0.018	0.006

^a Switching frequencies (per cell per generation) were determined by best fit to model curves generated by using equation 2 (see Materials and Methods).

The use of *fimA-lacZYA* fusions to study the *fim* switch eliminates this problem. However, the outgrowth of Lac⁻ (switch-off) over Lac⁺ (switch-on) bacteria could still bias estimates of switching frequencies. Competition experiments were carried out with two strains, AAEC374A (*fimB* *fimE* *fimA-lacZYA*, switch locked on) and AAEC376A (*fimB*

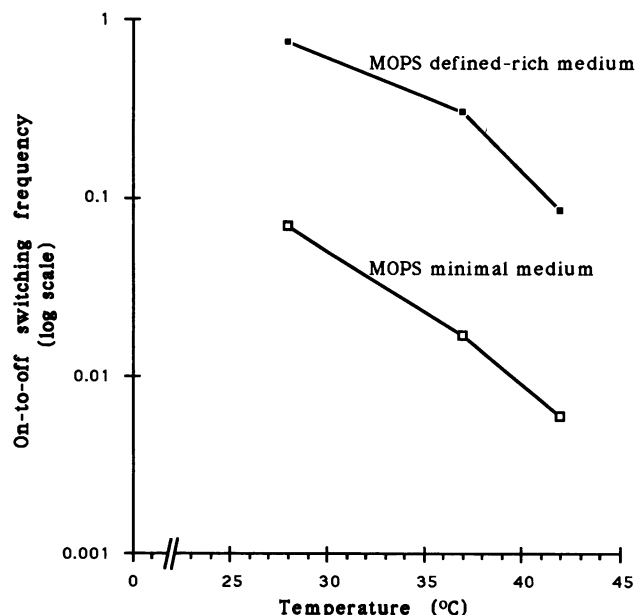


FIG. 3. Comparison of on-to-off (*fimE*-dominated) switching frequencies (per cell per generation) obtained with different temperatures and media in AAEC198A (*fimB*⁺ *fimE*⁺ *fimA-lacZYA*).

TABLE 4. *fimB*-promoted (*fimB*⁺ *fimE*) switching frequencies in liquid media at different temperatures

Medium and type of switching	Switching frequency ^a (10 ⁻⁴) at:			
	28°C	37°C	40°C	42°C
Defined-rich				
On to off	4.2 ± 0.8 ^b	15.0 ± 8.0	NT ^c	2.7 ± 1.7
Off to on	2.3 ± 1.4	11.0 ± 1.0	10.0 ± 3.0	4.2 ± 0.6
Minimal				
On to off	6.9 ± 2.6	11.0 ± 2.4	NT	3.4 ± 2.8
Off to on	3.9 ± 1.6	24.0 ± 11	13.0 ± 4.0	4.2 ± 2.6

^a Switching frequencies (per cell per generation) were determined by using equation 1 (see Materials and Methods).

^b 95% confidence interval calculated by using the *t* distribution.

^c NT, not tested.

fimE fimA-lacZYA, switch locked off), to test for differential growth. In all but one situation, outgrowth did not significantly alter the ratio of Lac⁺ to Lac⁻ bacteria. In minimal medium at 28°C, the growth rate of Lac⁻ bacteria (switch off) was 7% faster than that of Lac⁺. To determine the switching frequency under this condition, the rate of outgrowth was subtracted from the observed switching frequency (data not shown).

Switching frequencies in liquid media: *fimB*⁺ *fimE*. To measure easily *fimB*-promoted switching, the overriding effect of *fimE* was removed. Consequently, *fimB*-promoted switching in *fimB*⁺ *fimE* *fimA-lacZYA* strains (AAEC370A and -372A) was determined. *fimB*-promoted switching was measured in both directions, at different temperatures, and in both rich and minimal media (Table 4). Like *fimE*-promoted switching, *fimB*-promoted switching was affected by temperature and to a lesser extent by media. Unlike *fimE*-promoted activity, *fimB* activity increased between 28 and 37°C but declined at 42°C. In rich medium, the optimal temperature for *fimB*-promoted off-to-on switching was between 37 and 41°C, whereas the optimum appeared to be 37°C in minimal medium (Fig. 4). *fimB*-promoted switching was lower in defined rich than in minimal medium, in direct contrast to *fimE*-promoted switching, which increased over 10-fold.

Comparing *fimE* and *fimB* for the ability to promote switching, it is evident that *fimE* has an overriding effect in rich medium conditions, especially at lower temperatures (Tables 2 and 3). The optimal conditions for *fimB*-promoted switching are those that significantly slow *fimE*-promoted switching. These opposing responses amplify the effect on the switch such that *fimB*-promoted activity becomes important in the wild type (*fimB*⁺ *fimE*⁺) in minimal medium at temperatures between 37 and 41°C. The switch-on equilibria for the different conditions can be calculated by dividing the switch turn-on frequency by the total switching frequency (Fig. 5). This represents the proportion of bacteria that contain the switch in the on orientation once equilibrium is reached.

Addition of aliphatic amino acids to minimal medium stimulates switching. Lrp has been shown to control the expression of a regulon that includes Pap, K99, and type 1 fimbriae (4, 7). Whereas K99 expression is repressed by alanine and leucine, expression of Pap fimbriae is apparently unaffected by these amino acids (7). We have demonstrated previously that *lrp* stimulates both *fimE*- and *fimB*-promoted switching in leucine-replete defined rich medium (4).

Since both *lrp* and media (Tables 2 to 4) clearly influence

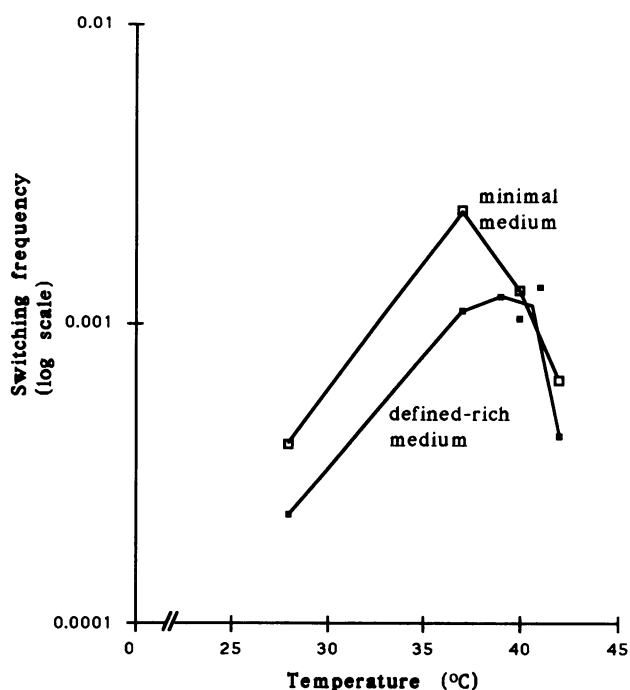


FIG. 4. Effects of temperature and media on *fimB*-promoted on-to-off switching frequencies (per cell per generation).

the *fim* switch, the effect of aliphatic amino acids on switching was investigated. The addition of leucine to minimal medium creates an isoleucine-valine auxotrophy in *E. coli* K-12, and consequently these two amino acids must also be

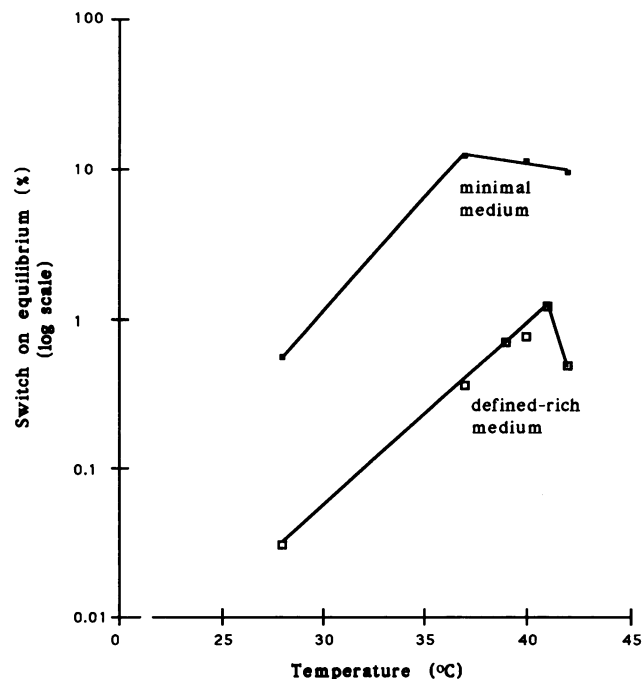


FIG. 5. Equilibrium (on) of the *fim* switch with different temperatures and growth media. Values (per cell per generation) were calculated by dividing the frequency of turnon by the total switching frequency.

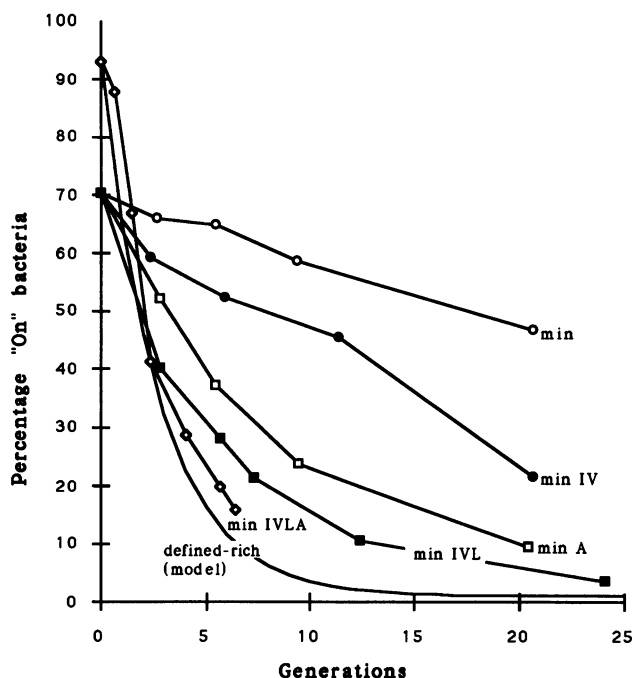


FIG. 6. Effect of the aliphatic amino acids A, I, L, and V on the frequency of on-to-off switching in AAEC198A (*fimB*⁺ *fimE*⁺ *fimA-lacZYA*). Frequencies are shown in Table 5.

added together with leucine. To test the effect of these amino acids on *fimE* activity, switching frequencies were determined for AAEC198A (*fimB*⁺ *fimE*⁺ *fimA-lacZYA*) in supplemented minimal medium (Fig. 6 and Table 5). The addition of I, V, L, and A to minimal medium increased (15-fold) the frequency of *fimE*-promoted switching to that measured in defined rich medium (Table 5). The additions of isoleucine and valine alone and of isoleucine, valine, and leucine increased on-to-off switching three- and eightfold, respectively. The addition of exogenous alanine increased on-to-off switching sixfold. To establish whether stimulation by these aliphatic amino acids requires *lrp*, on-to-off switching frequencies in an *lrp* mutant (BGEC053) in defined rich and minimal media were determined. In the absence of *lrp*, little difference in on-to-off switching was detected between the two media (1.4×10^{-4} versus 1.9×10^{-4} , respectively). Thus, stimulation of *fimE*-promoted switching by these aliphatic amino acids requires *lrp*.

Like *fimE*-promoted switching, *fimB*-promoted switching is stimulated by aliphatic amino acids. Off-to-on switching increased fourfold when I, V, L, and A were added to

minimal medium. However, in stark contrast to that of *fimE*-promoted switching, the frequency of *fimB*-promoted switching was eightfold higher in minimal medium with I, V, L, and A than in defined rich medium (Table 5). Therefore, additional responses to growth in defined rich medium cause a specific reduction in the frequency of *fimB*-promoted switching, at least in the off-to-on direction. Again, to determine the contribution of *lrp* to these medium-dependent effects, *fimB*-promoted switching was measured for *lrp* mutant BGEC081 in defined rich medium (2×10^{-5}), minimal medium (4×10^{-5}), and minimal medium with I, V, L, and A (3×10^{-5}). These results confirm that increased switching in minimal medium with I, V, L, and A requires *lrp*. Moreover, they show that *lrp* appears to differentially regulate *fimB*- and *fimE*-promoted switching in defined rich medium.

DISCUSSION

Until recently, *E. coli* K-12 strains studied as wild types contained insertion sequence-derived mutations in *fimE* (6). In contrast to slowly switching (10^{-3} to 10^{-4}) *fimE* mutants, wild-type strains (*fimB*⁺ *fimE*⁺) were known to switch off far more rapidly (4, 6), even though accurate determination of wild-type switching frequencies was not possible. By varying growth conditions, we have now developed an assay to measure wild-type switching frequencies precisely. Here it is demonstrated that the *fim* switch in *E. coli* K-12 strain MG1655 is controlled by environmental factors, including temperature and growth media. Switching promoted by both *fimB* and *fimE* is shown to be controlled. *fimE*-promoted switching can occur remarkably rapidly (0.75 per cell per generation), and hence the synthesis of type 1 fimbriae can be shut down abruptly.

We have shown previously that switching promoted by both *fimB* and *fimE* is stimulated in rich, amino acid-replete medium by Lrp (4). Here we show that switching promoted by both *fimB* and *fimE* is stimulated by the addition of exogenous alanine, leucine, and isoleucine plus valine to minimal medium. A lower frequency of *fimE*-promoted on-to-off switching in minimal versus rich medium (over 10-fold) can be accounted for by the presence of I, V, L, and A in defined rich medium and the likely interaction of these amino acids with Lrp. In support of this hypothesis, the frequencies of *fimE*-promoted switching were similar in both media (10^{-4}) in the absence of *lrp*.

Paradoxically, *fimB*-promoted switching is slightly more rapid in minimal than in defined rich medium, despite the fact that the addition of I, V, L, and A to minimal medium stimulates switching. *fimB*-promoted switching, in an *lrp* mutant, is reduced to similar levels in defined rich and minimal media and minimal medium with I, V, L, and A. Thus, some factor present in defined rich medium, but

TABLE 5. Switching frequencies in minimal medium supplemented with aliphatic amino acids

Inversion type	Switching frequency ^a in medium				
	Min ^b	Min + A	Min + I + V	Min + I, V, L	Min + I, V, L, A
<i>fimE</i> promoted ^c	0.018	0.11	0.05	0.15	0.28
<i>fimB</i> promoted ^d	$2.4 \pm 1.1 (10^{-3})^e$	NT ^f	NT	NT	$8.9 \pm 3.0 (10^{-3})$
					$1.1 \pm 0.2 (10^{-3})$

^a Per cell per generation.

^b Min, minimal.

^c *fimE*-promoted switching was determined by best fit to modeled switching frequencies calculated by using equation 2 (see Materials and Methods).

^d *fimB*-promoted switching frequencies were calculated by using equation 1 (see Materials and Methods).

^e 95% confidence interval calculated by using the *t* distribution.

^f NT, not tested.

absent or reduced in minimal medium, presumably blocks the ability of Lrp and I, V, L, and A to stimulate *fimB*-promoted switching. *lrp* appears to differentially regulate *fimE*- and *fimB*-promoted switching in defined rich medium.

Over 30 years ago, Brinton showed that on-to-off fimbrial phase variation declines at higher temperatures (8). Although the frequencies of phase transition were very low and complicated by differential growth rates, these data provided evidence for environmental control. We have studied the influence of temperature on the *fim* switch in detail. Whereas *fimE*-promoted switching declines as temperature increases, *fimB*-promoted switching actually increases to optima between 37 and 41°C.

Temperature control of the *fim* switch could operate at many levels. However, since on-to-off switching promoted by *fimB* and *fimE* shows inverse responses to temperatures below 40°C, it seems unlikely that the recombination, leading to inversion of the *fim* switch, is affected directly by temperature. The decline in *fimB*-promoted activity above 40°C could reflect thermal instability of either FimB or an additional factor required for its activity. Presumably, temperature control could operate at the level of expression of *fimB* and *fimE*. The *fim* switch is affected by *hns*, at least in a *fimE* mutant background (21, 26, 45). H-NS has been implicated in the thermal control of other systems (9, 19, 25, 30, 46), and it is therefore possible that this protein regulates transcription of *fimB* and *fimE* in response to temperature. Preliminary studies demonstrate that transcription of both *fimB* and *fimE* is strongly enhanced in an *hns* mutant at 37°C (5). Further studies will characterize the effects of *hns* and temperature on the *fim* switch.

It has long been known that growth conditions can affect the proportion of fimbriate bacteria (8, 12, 29, 35, 36). Growth in poorly aerated static liquid medium enriches for fimbriate bacteria, whereas growth in well-aerated liquid medium, or on agar, favors the afimbriate phase. It has been clearly shown that at least some of these effects reflect strong selection for or against fimbriate bacteria. Several authors have suggested that liquid and agar represent different environments that result in differential regulation of the *fim* switch (22, 43). We have measured switching frequencies both on agar and in liquid media and found no obvious differences in control.

One of the most striking observations is the discrepancy between relatively slow *fimB*-promoted switching and much more rapid *fimE*-promoted switching. The molecular basis for this difference and the biological implications are the subjects of further work. We have shown previously that *fimB* and *fimE* appear to be transcribed at comparable levels (4). Therefore, it seems likely that either the translation, stability, or specific activity of FimE is substantially greater than that of FimB.

The capacity of the *fim* switch to turn off rapidly has several important implications. First, in analysis of clinical isolates, the fimbrial phase has often been assayed after growth on rich agar medium. From our data, it is clear that rapid turnover under such conditions invalidates this type of analysis. Second, the ability of *E. coli* to become afimbriate quickly may be of pathogenic significance (31). Our data clearly show that the *fim* switch can turn off extremely rapidly under appropriate conditions. Moreover, preliminary results (5) suggest that fimbriae can be lost from the bacterial surface within a few generations of the *fim* switch turning off.

What do our results suggest about the regulation of type 1 fimbriation? The observed temperature control implies that synthesis of type 1 fimbriae is repressed outside the host but

has the potential to switch on in the host. Moreover, control of the *fim* switch by levels of aliphatic amino acids (alanine, isoleucine, leucine, and valine) suggests that expression could vary in different host compartments.

ACKNOWLEDGMENTS

This work was supported in part by intramural support (1-130-400-0040) from the Bowman Gray School of Medicine to I.C.B., postdoctoral fellowship support from Eli Lilly and Company to D.L.G., and Public Health Service research grant AI24734 to B.I.E. from the National Institutes of Health.

We thank R. Matthews, S. Richardson, D. Wozniak, and I. van de Rijn for helpful comments and advice. We thank M. McClain for critical reading of the manuscript.

REFERENCES

1. Abraham, J. M., C. S. Freitag, J. R. Clements, and B. I. Eisenstein. 1985. An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 82:5724-5727.
2. Abraham, J. M., C. S. Freitag, R. M. Gander, J. R. Clements, and B. I. Eisenstein. 1986. Fimbrial phase variation and DNA rearrangements in uropathogenic isolates of *Escherichia coli*. *Mol. Biol. Med.* 3:495-508.
3. Bloch, C. A., and P. E. Orndorff. 1990. Impaired colonization by and full invasiveness of *Escherichia coli* K1 bearing a site-directed mutation in the type 1 pilin gene. *Infect. Immun.* 58:275-278.
4. Blomfield, I. C., P. J. Calie, K. J. Eberhardt, M. S. McClain, and B. I. Eisenstein. 1993. Lrp stimulates phase variation of type 1 fimbriation in *Escherichia coli* K-12. *J. Bacteriol.* 175:27-36.
5. Blomfield, I. C., and B. I. Eisenstein. Unpublished data.
6. Blomfield, I. C., M. S. McClain, J. A. Princ, P. J. Calie, and B. I. Eisenstein. 1991. Type 1 fimbriation and *fimE* mutants of *Escherichia coli* K-12. *J. Bacteriol.* 173:5298-5307.
7. Braaten, B. A., J. V. Platko, M. W. van der Woude, B. H. Simons, F. K. de Graaf, J. M. Clavo, and D. A. Low. 1992. Leucine-responsive regulatory protein controls the expression of both *pap* and *fan* pili operons in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 89:4250-4254.
8. Brinton, C. C., Jr. 1959. Non-flagellar appendages of bacteria. *Nature (London)* 183:782-786.
9. Dagberg, B., and B. E. Uhlin. 1992. Regulation of virulence-associated plasmid genes in enteroinvasive *Escherichia coli*. *J. Bacteriol.* 174:7606-7612.
10. de Ree, J. M., and J. F. van den Bosch. 1987. Serological response to the P fimbriae of uropathogenic *Escherichia coli* in pyelonephritis. *Infect. Immun.* 55:2204-2207.
11. Dorman, C. J., and C. F. Higgins. 1987. Fimbrial phase variation in *Escherichia coli*: dependence on integration host factor and homologues with other site-specific recombinases. *J. Bacteriol.* 169:3840-3843.
12. Duguid, J. P., and R. R. Gillies. 1957. Fimbriae and adhesive properties of dysentery bacilli. *J. Pathol. Bacteriol.* 74:397-411.
13. Eisenstein, B. I. 1981. Phase variation of type 1 fimbriae in *Escherichia coli* is under transcriptional control. *Science* 214:347-349.
14. Eisenstein, B. I., and D. C. Dodd. 1982. Pseudocatabolite repression of type 1 fimbriae of *Escherichia coli*. *J. Bacteriol.* 151:1560-1567.
15. Eisenstein, B. I., D. S. Sweet, V. Vaughn, and D. I. Friedman. 1987. Integration host factor is required for the DNA inversion that controls phase variation in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 84:6506-6510.
16. Eisenstein, B. I., and V. Vaughn. Unpublished data.
17. Freitag, C. S., J. M. Abraham, J. R. Clements, and B. I. Eisenstein. 1985. Genetic analysis of the phase variation control of expression of type 1 fimbriae in *Escherichia coli*. *J. Bacteriol.* 162:668-675.
18. Gander, R. M., and V. L. Thomas. 1987. Distribution of type 1 and P pili on uropathogenic *Escherichia coli* O6. *Infect. Immun.* 55:293-297.

19. Goransson, M., B. Sonden, P. Nilsson, B. Dagberg, K. Forsman, K. Emanuelsson, and B. E. Uhlin. 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. *Nature* (London) **344**:682-685.
20. Guyer, M. S., R. R. Reed, J. A. Steitz, and K. B. Low. 1981. Identification of a sex-factor-affinity site in *E. coli* as gamma delta. *Cold Spring Harbor Symp. Quant. Biol.* **45**:135-140.
21. Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, I. R. Booth, G. May, and E. Bremer. 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli*. *Cell* **52**:569-584.
22. Hultgren, S. J., W. R. Schwan, A. J. Schaeffer, and J. L. Duncan. 1986. Regulation of production of type 1 pili among urinary tract isolates of *Escherichia coli*. *Infect. Immun.* **54**: 613-620.
23. Jann, K., G. Schmidt, E. Blumenstock, and K. Vosbeck. 1981. *Escherichia coli* adhesion to *Saccharomyces cerevisiae* and mammalian cells: role of piliation and surface hydrophobicity. *Infect. Immun.* **32**:484-489.
24. Johnson, J. R. 1991. Virulence factors in *Escherichia coli* urinary tract infection. *Clin. Microbiol. Rev.* **4**:80-128.
25. Jordi, B. J. A. M., B. Dagberg, L. A. M. de Haan, A. M. Hamers, B. A. M. van der Zeijst, W. Gaastra, and B. E. Uhlin. 1992. The positive regulator CfaD overcomes the repression mediated by histone-like protein H-NS (H1) in the CFA/I fimbrial operon of *Escherichia coli*. *EMBO J.* **11**:2627-2632.
26. Kawula, T. H., and P. E. Orndorff. 1991. Rapid site-specific DNA inversion in *Escherichia coli* mutants lacking the histone-like protein H-NS. *J. Bacteriol.* **173**:4116-4123.
27. Klemm, P. 1986. Two regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type 1 fimbriae in *Escherichia coli*. *EMBO J.* **5**:1389-1393.
28. Korhonen, T. K., and M. Rhen. 1982. Bacterial fimbriae as vaccines. *Ann. Clin. Res.* **14**:272-277.
29. Maccacaro, G. A., and W. Hayes. 1961. The genetics of fimbriation in *Escherichia coli*. *Genet. Res.* **2**:394-405.
30. Maurelli, A. T., and P. J. Sansonetti. 1988. Identification of a chromosomal gene controlling temperature-regulated expression of *Shigella* virulence. *Proc. Natl. Acad. Sci. USA* **85**:2820-2824.
31. May, A. K., C. A. Bloch, R. G. Sawyer, M. D. Spengler, and T. L. Pruett. 1993. Enhanced virulence of *Escherichia coli* bearing a site-directed mutation in the major structural subunit of type 1 fimbriae. *Infect. Immun.* **61**:1667-1673.
32. McClain, M. S., I. C. Blomfield, K. J. Eberhardt, and B. I. Eisenstein. 1993. Inversion-independent phase variation of type 1 fimbriae in *Escherichia coli*. *J. Bacteriol.* **175**:4335-4344.
33. McClain, M. S., I. C. Blomfield, and B. I. Eisenstein. 1991. Roles of *fimB* and *fimE* in site-specific DNA inversion associated with phase variation of type 1 fimbriae in *Escherichia coli*. *J. Bacteriol.* **173**:5308-5314.
34. Neidhardt, F. C., P. L. Bloch, and D. F. Smith. 1974. Culture media for enterobacteria. *J. Bacteriol.* **119**:736-747.
35. Old, D. C., I. Cornell, L. F. Gibson, A. D. Thomson, and J. P. Duguid. 1968. Fimbriation, pellicle formation and the amount of growth of salmonellas in broth. *J. Gen. Microbiol.* **51**:1-16.
36. Old, D. C., and J. P. Duguid. 1970. Selective outgrowth of fimbriate bacteria in static liquid medium. *J. Bacteriol.* **103**:447-456.
37. Orndorff, P. E., P. A. Spears, D. Schauer, and S. Falkow. 1985. Two modes of control of *pilA*, the gene encoding type 1 pilin in *Escherichia coli*. *J. Bacteriol.* **164**:321-340.
38. Pallesen, L., O. Madsen, and P. Klemm. 1989. Regulation of the phase switch controlling expression of type 1 fimbriae in *Escherichia coli*. *Mol. Microbiol.* **3**:925-931.
39. Rene, P., M. Dinolfo, and F. J. Silverblatt. 1982. Serum and urogenital antibody responses to *Escherichia coli* pili in cystitis. *Infect. Immun.* **38**:542-547.
40. Rene, P., and F. J. Silverblatt. 1982. Serological response to *Escherichia coli* pili in pyelonephritis. *Infect. Immun.* **37**:749-754.
41. Salit, I. E., J. Vavougiou, and T. Hofmann. 1983. Isolation and characterization of *Escherichia coli* pili from diverse clinical sources. *Infect. Immun.* **42**:755-762.
42. Schaeffer, A. J., W. R. Schwan, S. J. Hultgren, and J. L. Duncan. 1987. Relationship of type 1 pilus expression in *Escherichia coli* to ascending urinary tract infections in mice. *Infect. Immun.* **55**:373-380.
43. Schwan, W. R., H. S. Seifert, and J. L. Duncan. 1992. Growth conditions mediate differential transcription of *fim* genes involved in phase variation of type 1 pili. *J. Bacteriol.* **174**:2367-2375.
44. Silhavy, T. J., M. L. Berman, and L. W. Enquist. 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45. Spears, P. A., D. Schauer, and P. E. Orndorff. 1986. Metastable regulation of type 1 piliation in *Escherichia coli* and isolation and characterization of phenotypically stable mutants. *J. Bacteriol.* **168**:179-185.
46. Yamada, M., C. Sasakawa, N. Okada, S. I. Makino, and M. Yoshikawa. 1989. Molecular cloning and characterization of chromosomal virulence region *kcpA* of *Shigella flexneri*. *Mol. Microbiol.* **3**:207-213.